5-1990

Immunological Effects of Ketoconazole, Itraconazole and Fluconazole on Lymphocyte Cell Proliferation and Natural Killer Cell Activity in Immune-Normal, Cyclosporine-Compromised and Cyclophosphamide-Compromised Mouse Models

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IMMUNOLOGICAL EFFECTS OF KETOCONAZOLE, ITRACONAZOLE AND FLUCONAZOLE ON LYMPHOCYTE CELL PROLIFERATION AND NATURAL KILLER CELL ACTIVITY IN IMMUNE-NORMAL, CYCLOSPORINE-COMPROMISED AND CYCLOPHOSPHAMIDE-COMPROMISED MOUSE MODELS

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

Approved:

UTAH STATE UNIVERSITY
Logan, Utah
1990

ACKNOWLEDGEMENTS

I would like to thank Dr. Andy Anderson, chairman of my committee, for his support and advise and Dr. Reed Warren for serving as a member of my committee and for his help and suggestions in the immunological aspects of my research. I also would like to thank Dr. Stan Allen for his encouragement to pursue a masters degree and for his financial support and advice in regards to my research project.

I would also like to thank Roger Burger for his help with the immunological assays and for the use of the lab and Jeff Siems and Mary Quinn for their help. I would especially like to thank my wife, Carmalee, for all her support, encouragement and the sacrifices she made in order for me to continue my education.

Jeffrey Tullis Mitchell

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Immunological Effects of Ketoconazole, Itraconazole and Fluconazole on Lymphocyte Cell Proliferation and Natural Killer Cell Activity in Immune-Normal, Cyclosporine-compromised and Cyclophosphamide-compromised Mouse Models

by

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Utah State University, 1990

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Over the past several years there has been a steady increase in the incidence of immunologically compromised patients. This has been the result of both chemical agents, such as those used in cancer chemotherapy, and biological agents such as HIV, the cause of Acquired Immunodefeciency Syndrome (AIDS). The increase in immune-suppressed patients has lead to an increase in life-threatening mycoses requiring treatment with antifungal agents. Pharmaceutical companies have increased research for the development of new antifungal agents which are more effective and less toxic than those that are currently used. Several researchers have reported on antifungal agents that demonstrate both positive and negative effects on the immune system. Because antifungal therapy relies on host immune defenses in eliminating diseases, more emphasis is being placed on how antifungal agents interact with the immune system. The purpose of this study was to evaluate the effects of Ketoconazole, Itraconazole and Fluconazole on T- and B-cell proliferation and natural killer cell activity using normal, cyclosporine-compromised and cyclophosphamide-compromised immune models in mice. T and B cells obtained from the spleens of

Balb/c mice were mitogen stimulated and grown in the presence of 0, 1, 2, 4, 8 and 16 μg/ml of these 3 antifungal agents. Cell proliferation was determined by the uptake of ^{3}[H]-thymidine and was measured as counts per minute. Natural killer cell activity was measured by the release of 51-sodium chromate (^{51}Cr) into the supernatant by ^{51}Cr-labeled Yac cells. Ketoconazole caused a significant reduction in cell proliferation in all immune models in both T and B cells. Itraconazole also significantly inhibited cell proliferation in all models in both T and B cells as well as natural killer (NK) cell activity in the immune-normal model. Viability studies on mitogen-stimulated lymphocytes suggest that the inhibitory effects of Ketoconazole and Itraconazole on lymphocyte proliferation are due to toxic effects. Fluconazole appears to have few if any inhibitory effects on either cell proliferation or natural killer cell activity.

(65 pages)

INTRODUCTION

Over the past several years the incidence of life-threatening fungal infections has been on the rise. This can be attributed to organ transplantation, chemotherapy for the treatment of cancer and the AIDS epidemic. The first heart transplant was performed almost 20 years ago. The patient died 18 days later because of an infection that his immune system was unable to eliminate. This was a result of immunosuppressive therapy used to fight rejection of the heart (9). Despite the advent of cyclosporine in the late 1970s, infections resulting from immunosuppression are still a problem. Cancer chemotherapy using such drugs as cyclophosphamide also has a significant impact on the immune system, and many cancer patients die from the infections brought about by the immunosuppression rather than from the cancer itself. Over the past few years the AIDS epidemic has increased at an alarming rate. The Centers for Disease Control in Atlanta reported that as of July, 1989 over 100,000 cases of AIDS had been reported and over 59,000 AIDS-related deaths had occurred (13). All of these factors have resulted in the increase of diseases that have rarely been seen or have not been considered life threatening. Antifungal therapy has not been a field of great research intensity but is becoming of much greater importance as the need for new and more effective agents has become evident (14). Intervention in the disease process by an antimicrobial agent still relies heavily on the host immune system to aid in combating and clearing infections. In antifungal therapy, host immunity is even more important because most antifungal agents are fungistatic and not fungicidal. The importance of developing antimicrobial agents that do not impair the immune system is growing, especially for patients whose immune systems are already impaired. The objective of this research was to study the effects of Ketoconazole, a currently used antifungal agent, and 2 new compounds that are now in clinical trials, Fluconazole and Itraconazole, in both immune-normal and immune-compromised models.

REVIEW OF LITERATURE

Fungal Infections and Host Immunity

In order for fungal organisms, the majority of which are opportunistic pathogens, to cause disease they must evade elimination by the immune system. It is generally agreed that cell-mediated immunity (CMI) is one of the most effective mechanisms of resistance to fungal infections (8,21). Mechanisms against such organisms as *Candida albicans* are believed to involve specific T cells (8). Evidence supporting these beliefs lies in the fact that patients having recurrent fungal infections often display defective delayed hypersensitivity, as demonstrated with skin tests. Patients with AIDS who lack certain T-cell functions often have fungal infections such as candidiasis and cryptococcosis. Once specific T cells have been activated, they produce soluble factors which stimulate non-specific immunity via polymorphonuclear leukocytes (PMN) and mononuclear phagocytes, which play an important role in eliminating the organisms from the host. Cell-mediated immunity, in particular natural killer cell activity, is believed to be very important against *Cytococcal* infections (21). It is when these lines of defense are broken down, through either chemical or biological means, that serious and potentially fatal fungal infections can occur.

Immunosuppressive Agents

As stated earlier, several drugs frequently used in cancer therapy and organ transplantation produce a marked suppressive effect on the immune system. Two common drugs used for these purposes are cyclophosphamide and cyclosporine.

Cyclophosphamide. Cyclophosphamide is used in the treatment of several malignancies including malignant lymphomas, multiple myeloma and leukemias. It interferes with the growth of susceptible neoplasms and, to some extent, normal tissues, although the mechanism of action of this drug is not known. One of the expected effects of cyclophosphamide treatment is leukopenia which is used as a guide to therapy. Therapy includes an intravenous induction dose of 40-50 mg/kg and then either daily doses of 1-5 mg/kg or weekly doses of 10-15 mg/kg (3).

An interesting effect that cyclophosphamide can have under certain conditions is to actually increase resistance to infections. Francesco Bistoni and his associates performed several experiments with cyclophosphamide *in vivo* and *in vitro* and found that for the first 6-10 days after treatment with cyclophosphamide, there was a significant increase in susceptibility to infection followed by a period from 12-24 days after treatment in which there was a significant rise in resistance to infection by *Candida* species (7). They found the resistance was due to, at least in part, a lymphocyte with exceptional killing abilities toward *Candida* species which was clearly distinct from NK cells. There was also an increase in the NK-cell lytic activity as well as above normal numbers of peripheral blood PMN's.

Cyclosporine. Cyclosporine is a cyclic polypeptide immunosuppressive drug which is produced as a metabolite of the fungus species *Tolypocladium inflatum* Gams. It is a potent agent that suppresses cell-mediated reactions such as allograft rejection, delayed hypersensitivity and experimental allergic encephalomyelitis. The mechanism of action is not known at this time, but experimentation suggests that cyclosporine interferes with the G_0 or G_1-phase of cell growth in immunocompetent lymphocytes. The lymphocytes that seem most affected by cyclosporine are the T-helper cells and to a lesser extent T-suppressor cells. Cyclosporine also appears to inhibit production and release of lymphokines including interlukin-2 (T cell growth factor) (4). Because of the potency of the drug it is only to be used by physicians with experience in immunosuppressive therapy with the patients being closely monitored for toxicity. Ketoconazole and Amphotericin B increase plasma levels of cyclosporine by decreasing metabolism of the drug (5).

Antifungal Agents

In cases of life-threatening fungal infections, Amphotericin B is usually the drug of choice, even though the drug itself is highly toxic. Patients receiving the drug may develop permanent kidney damage (5). Pharmaceutical companies are now developing compounds which will provide an alternative means for treating life-threatening mycosis with fewer or less severe toxic side effects. Ketoconazole is a more recently developed antifungal agent that can be used as an alternative to

Amphotericin B in some cases. Itraconazole and Fluconazole are compounds that are now in clinical trials and offer much hope as an alternative to treatment with Amphotericin B.

Ketoconazole. Ketoconazole is an antifungal agent that falls into the class known as imidazoles. The effects of Ketoconazole on fungi are primarily fungistatic. Ketoconazole, for the treatment of candidiasis, is given as a single daily dose of 200 mg with treatment lasting as short as 1 week and can be as long as 4 weeks or more in dermatophytic infections. Ketoconazole reaches a mean peak plasma concentration of approximately 3.5 μg/ml within 1 to 2 hours after administration. It is indicated in the treatment of the following infections: candidiasis, chronic mucocutaneous candidiasis, oral thrush, candiduria, blastomycosis, coccidioidomycosis, histoplasmosis, chromomycosis and paracoccidioidomycosis. Hepatoxicity occurs in about 1:10,000 patients, and there have been a few cases of death related to treatment with Ketoconazole (6).

Although the mechanism of action of Ketoconazole is not precisely known, experimental results indicate that it prevents the demethylation of lanosterol in fungi (10,12,18,24). Lanosterol is the precursor to ergosterol, which is the major membrane sterol in fungi. Without ergosterol the cell's permeability increases, which inhibits growth of the organism (12,17). In mammalian cells Ketoconazole has also been shown to inhibit the demethylation of lanosterol, which in turn inhibits cholesterol synthesis. One theory for the inhibitory effects which Ketoconazole has on the immune system *in vitro* is that most cells are able to use exogenous cholesterol acquired from serum, but certain cells, such as lymphocytes, require endogenous cholesterol prior to proliferation (12).

Thomas Buttke and Stanley Chapman performed experiments to determine the effect of Ketoconazole on mitogen-induced DNA synthesis and cholesterol biosynthesis in lymphocytes (12). Human and mouse spleen cells were stimulated by phytohemaglutinin (PHA), *Staph aureus* or lipopolysaccharide (LPS) and were incubated in the presence of various concentrations of Ketoconazole. During the last 16 to 18 hours of incubation, mitogen-induced DNA synthesis was measured by the addition of 0.5 μCi of tritiated thymidine. At the end of the incubation period the cells were harvested on glass fiber filters, and the amount of incorporated tritiated thymidine was

measured with a scintillation counter. At Ketoconazole concentrations of 10 μg/ml, DNA synthesis by lymphocytes was 80-90% inhibited. Sterol synthesis was measured by incubating mitogen-stimulated human and mouse lymphocytes for 24 hours followed by the addition of 10 μCi of [^{14}C]acetate and continuing incubation for an additional 6 hours. Lipids were extracted from the cells and were quantified by liquid scintillation counting. It was shown that at concentrations of 1 μg/ml, well within therapeutic levels, 80-90% inhibition of cholesterol synthesis was observed.

In cell-proliferation experiments very similar to those of Buttke, D.S. Senior and J.T.B. Shaw at Pfizer Labs demonstrated that Ketoconazole has an inhibitory effect on blastogenesis of lymphocytes at levels as low as 1 μg/ml (22). They also performed experiments to ascertain the effects of Ketoconazole on the *Candida*-killing activity of PMN's using a radiolabel release assay. *Candida albicans* blastospores were radiolabeled with ^{3}H-uridine for 60 minutes and were then incubated in the presence of human PMN leukocytes at a target:effector cell ratio of 3:1. Following a 60-minute incubation, the PMN's were lysed with sodium deoxycholate to release label from engulfed blastospores. Supernatant from the cultures was counted on a liquid scintillation counter to determine the extent of candida blastospore lysis. Ketoconazole caused significant reductions in blastospore killing at 10 μg/ml.

Studies were conducted at the Merck Institute for Therapeutic Research to determine the effect of antifungal agents on the chemiluminescence (CL) response (1,2). Mouse spleen cells were collected and adjusted to 1 X 10^{7} cells/ml and 470 μl were aliquoted into lumacuvettes. After the addition of 10 μl of known concentrations of various antifungal agents, including ketoconazole, the cells were incubated for 30 minutes at 37C. After this incubation, 10 μl of luminol (1 mg/ml) was added, and the chemiluminescence response was measured every 20 seconds for 30 minutes. It was found that at concentrations as low as 3 μg/ml, Ketoconazole inhibited the CL response. The CL response measures light emitted by phagocytic cells upon producing oxygenated compounds, such as hydrogen peroxide and superoxide, during the respiratory burst.

D.J. Marmer and his colleges studied the effects of Ketoconazole on lymphocyte cell viability, chemotaxis, and killing by chemiluminescence (19). Cells that were obtained from donors receiving antifungal therapy at times corresponding to peak plasma levels or cells that were pretreated with antifungal agents were used in the various assays. Neutrophils treated with Ketoconazole were placed in wells, cut in an agarose plate, and tested for their chemotactic response to zymosan-activated autologous serum. After a 2 hour incubation, the plates were stained and assessed for the number of cells that migrated in a single plane. Experiments similar to those performed by the Merck Institute were carried out to determine the CL response. Neutrophils exposed to various concentrations of Ketoconazole for 3 hours were assessed for viability by exclusion of trypan blue. Phagocytic bactericidal activity was determined by incubating effector cells with *Staphylococcus aureus*, after which the bacteria were plated out and colony counts were performed. Ketoconazole was found to have no effect on any of the parameters measured. Some of these findings are in direct conflict with those reported earlier, which indicates possible differences in procedures or differences in assays performed. Most experimental evidence support the conclusion that Ketoconazole has some negative effect on the immune system.

Johnson et al. examined the effect of Ketoconazole on phagocytosis and killing of *C. albicans*, as well as germ tube elongation of ingested blastospores using human neutrophils (17). The parameters were assayed using 3 different tests in which: 1) Drug was added simultaneously to the phagocytes and blastospores, 2) Phagocytes were pretreated with drug and 3) Blastospores were pretreated with drug. Germ tube formation was measured at 2, 4 and 6 hours using a micrometer eyepiece. It was found that Ketoconazole has no significant effect on phagocytosis or the killing of *C. albicans*. Ketoconazole did not effect germ tube formation of either ingested or non-ingested blastospores in strains of *C. albicans* that were resistant to azoles, but had marked reduction in germ tube lengths in blastospores sensitive to azoles.

Van Rensburg and associates at the University of Pretoria, South Africa performed several experiments to determine the effect of Ketoconazole on neutrophil random migration, chemotaxis,

phagocytosis, and post-phagocytic hexose monophosphate shunt activity *in vitro* (25). Neutrophils were obtained from heparinized blood of 6 adult subjects at 2 and 96 hours after initiation of treatment, consisting of 200 mg of Ketoconazole twice daily. Ketoconazole caused an increase in chemotaxis in neutrophils incubated in the presence of either serum activated with bacterial endotoxin or synthetic chemotactic tripeptide at 2 hours after treatment. However, this increase was not observed at 96 hours after initiation of treatment. Ketoconazole did not affect the phagocytic activity of neutrophils incubated in the presence of *C. albicans*. Likewise, Ketoconazole had no effect on hexose monophosphate shunt (HMS) activity which was measured by the metabolism of D-[1-^{14}C] glucose labeled at the C-1 position.

Gergely et al. studied the effect of Ketoconazole on mitogen-induced blastogenesis, antibody-dependent cytotoxicity, natural-killer cell activity and the random migration of human leukocytes (16). Human peripheral blood lymphocytes were obtained from human donors. Ketoconazole reduced the uptake of ^{3}[H]thymidine by lymphocytes stimulated with phytohemaglutinin at levels as low as 1 μg/ml. At concentrations greater than 1 μg/ml, Ketoconazole decreased antibody-dependent cell-mediated cytotoxicity of human mononuclear cells against ^{51}Cr-labelled, antibody-coated chicken red blood cells. Ketoconazole at levels greater than 1 μg/ml also decreased natural killer-cell activity against ^{51}Cr-labelled K 562 target cells. Ketoconazole also reduced the amount of migration of leukocytes at levels as low as 1 μg/ml.

Itraconazole. Itraconazole is a new antifungal agent currently being tested by Janssen Pharmaceuticals, the same company that manufactures Ketoconazole. It is classified as a triazole antifungal agent. Preliminary indications are that it might be more effective and less toxic than Ketoconazole (10,15). It has the relatively long half-life of 1 day in man with peak plasma levels, following a 100 mg oral dose ranging from 0.2 - 0.6 μg/ml, although levels in tissues can be up to 14 times the levels achieved in plasma (15). Like Ketoconazole, Itraconazole's mode of action lies in its ability to block the cytochrome P-450 dependent demethylation of lanosterol (10). Janssen has reported that this inhibition occurs in fungi at extremely low (nM) concentrations. Patients receiving

up to 400 mg/day for 3 months had no increases in cholesterol levels. Other studies, performed with rats, indicate that levels as high as 40 mg/kg do not effect cytochrome P-450 dependent cholesterol synthesis in liver microsomes (10). Because it is a relatively new compound, little research has been done to evaluate its effect on the immune system. In the same experiments performed by the Merck Institute for Therapeutic Research with Ketoconazole, Itraconazole was determined to inhibit the CL response at 20 μg/ml, which is about 30 times higher than plasma levels and 2-3 times more than levels achieved in tissue (1). In the previously described experiments performed by Johnson, Itraconazole showed no significant effect on phagocytosis or killing of *C. albicans* blastospores (17).

Fluconazole. Fluconazole is a newly synthesized antifungal agent with a bis-triazole structure. The drug was developed and is currently being tested by Pfizer. Like other imidazole-related compounds, the mode of action appears to be the prevention of the C14 demethylation of lanosterol, which is a precursor for ergosterol, the major sterol in fungal membranes (20). The compound is one of the few antifungal agents that is water soluble, and is therefore capable of being administered orally or parentally. It has a half-life of 25 hours in man. Peak serum levels of approximately 1 μg/ml are achieved about 1 hour after administration of a 50 mg oral dose. (11). As with Itraconazole, little has been done to evaluate the effects of Fluconazole on the immune system. In the same studies discussed earlier, researchers at the Merck Institute for Therapeutic Research determined that at levels up to 20 μg/ml, Fluconazole had no significant effect on the CL response in mouse spleen cells (1). In the previously discussed experiments performed by Pfizer Central Research, which compared Ketoconazole with Fluconazole in mouse lymphocyte proliferation and *Candida* blastospore destruction, they found Fluconazole to have no significant effect in either of the 2 assays performed. Ketoconazole reduced proliferation and killing by human polymorphonuclear leukocytes (22).

Amphotericin B. As mentioned before, Amphotericin B is the drug of choice in life threatening circumstances. Amphotericin B is an antifungal agent developed by E.R. Squibb & Sons, Inc., Princeton, New Jersey. The antibiotic is derived from a strain of *Streptomyces nodosus*, and is

not soluble in water. The drug as supplied by the manufacturer, is solubilized by the addition of sodium desoxycholate. This forms a colloidal mixture upon the addition of water which allows it to be given parenterally. This compound has activity against a wide range of fungi with *in vitro* MIC values between 0.03 and 1.0 μg/ml. Amphotericin B is either fungistatic or fungicidal depending upon the levels achieved in body fluids and upon the susceptibility of the organism. The probable mode of action of Amphotericin B is binding to membrane sterols which creates a change in the membrane permeability and results in leakage. Mammalian cells also contain sterols in their membranes, and it is hypothesized that toxicity to human cells is caused by a similar mechanism. Treatment is started with an infusion of 1 to 5 mg of Amphotericin B per day, which is gradually increased to 0.65 mg/kg daily, with resultant peak plasma levels of 2 to 4 μg/ml. These levels can be maintained due to the relatively long half-life of 24 hours. The drug is eliminated very slowly by the kidneys with detectable levels in the urine for up to 7 weeks after discontinuation of treatment. There is little known concerning distribution into the tissues and metabolic pathways. Amphotericin B is recommended only for patients with progressive and potentially fatal fungal infections such as cryptococcosis, disseminated candidiasis, coccidioidomycosis, and histoplasmosis. Due to the many adverse reactions that frequently occur among those receiving treatment, the use of the drug is limited. The most commonly occurring reactions are: fever, headache, anorexia and weight loss. Although not as common, damage to the liver and kidneys, which may be permanent, can occur. For these reasons it is only administered in hospitals where appropriate monitoring for adverse side effects is provided. Until recently, Amphotericin B was about the only option in the case of life-threatening fungal diseases (5).

In the experiments performed by Abruzzo et al. on the effects of antifungal agents on the CL response, Amphotericin B was found to significantly reduce the CL response at a level of 1.25 μg/ml (1,2). In the previously described experiments by Johnson, Amphotericin B impaired phagocytosis in 1 of the 2 donors studied (17). Pre-treatment of blastospores showed no significant increase in killing. In addition to Ketoconazole, Marmer and his colleagues studied the effects of Amphotericin

B and Amphotericin B methyl ester, a water soluble derivative of Amphotericin B, on neutrophil functions both *in vivo* and *in vitro* (19). Amphotericin B and Amphotericin B methyl ester significantly inhibited migrating neutrophils and the CL response in incubations longer than 15 minutes and had no effect on phagocytosis or intracellular killing of bacteria. Tarnvik and Ansehn, from the Medical School at Linkoping, Sweden, evaluated the effects of Amphotericin B on lymphocyte stimulation (23). Lymphocytes were obtained from the venous blood of healthy donors and were adjusted to a concentration of 1 X 10^6 cells/ml. The lymphocytes were stimulated by the addition of phytohemagglutinin (PHA), concanavalin A (con A), pokeweed mitogen (PWM), and purified protein-derivative tuberculin (PPD). They were then incubated at 37C for 3 to 4 (PHA, con A) or 6 to 7 (PPD, PWM) days. Mixed lymphocyte cultures were also prepared by adding equal numbers of lymphocytes from 2 different individuals and incubating them for 6 to 7 days without the addition of any stimulants. Four hours before the end of incubation 0.4 μCi of ^{3}[H]-thymidine was added to each culture. The cultures were washed onto glass fiber filters, placed in 10 ml scintillation fluid and counted on a scintillation counter. Concentrations at and above 4 μg/ml of Amphotericin B caused significant reductions in the incorporation of tritiated thymidine irrespective of the stimulant used.

Vecchiarelli and colleagues at the University of Perugia, Perugia, Italy performed several experiments to evaluate the *in vitro* and *in vivo* effects of Amphotericin B on mouse spleen cells (26). In one experiment 8 week-old CD2F1 mice were given a single 10 mg/kg dose of Amphotericin B at 1, 2, 4, 6, 8 or 12 days prior to an intravenous challenge of 1 X 10^6 cells of *C. albicans* in 0.5 ml. Ten animals were challenged in each group which included non-treated controls. All animals died in the non-treated control group and in the 12 day prior-to-challenge group with mean survival times of 4.5 and 5 days respectively. Only 1 animal died in the 1 day prior-to-challenge group with a mean survival time of > 60 days. Two animals died in the 2 and 4 day prior-to-challenge groups, both with mean survival times of > 60 days. In the 6 and 8 day prior to challenge groups 3 animals died, with mean survival times of > 60 days. Vecchiarelli stated that these experimental results demonstrated

significant levels of protection against *C. albicans* infections with doses of Amphotericin B at time periods that could not be expected to offer any direct chemotherapeutic effects. This may not be the case as you consider the half-life of the drug being 24 hours and treatment of human subjects at 0.65 mg/kg results in serum levels of 2 to 4 μg/ml. These animals were treated with fifteen times the dose normally given to humans and therefore, even after allowing a faster rate of metabolism of the drug in mice, it is possible to have a therapeutic level of drug in the serum for several days. You must also take into account the fact that this treatment is for prophylaxis of the disease and not to cure it. In many cases the level of drugs needed to prevent a disease are much lower than the level needed to cure the same disease. In order to elucidate the effects behind these results, additional experiments were carried out *in vitro* to determine the effect of Amphotericin B on various effector-cell populations. Effector cells were fractionated by plastic adherence, nylon column adherence, carbonyl-iron powder and magnet, treatment with anti-theta (anti-Thy 1.2) serum containing complement and treatment with anti-asialo GMI serum with complement. These procedures resulted in distinct effector-cell populations with which to study the effect of Amphotericin B on specific effector-cell populations. In one experiment, 2 X 10^8 cells of *C. albicans* were labeled with 300 μCi of Na_2-$^{51}CrO_4$ for 2 hours at 37C. Various concentrations of effector cells were incubated with 5 X 10^4 cells of *C. albicans* for 4 hours, after which counts of chromium released into the supernatant were counted to determine the degree of lysis of the yeast. In a similar experiment, unlabeled yeast cells were incubated for 1 or 4 hours, plated out, and counted to determine the number of cells actually killed by the effector cells. Another experiment was performed in which phagocytic cells were incubated in the presence of 5 X 10^6 cells/ml of *C. ablicans* for various times. The cells were stained and the number of yeast cells phagocytized was determined. The *in vitro* results of the various fractionated cells obtained from animals dosed with 10 mg/kg 8 days before the assay, showed that the treated plastic adherent cells had an increase in activity of 2 to 4 times that of the untreated plastic adherent cells. Plastic nonadherent cells, nylon wool adherent cells and carbonyl-iron + magnet-treated cells all showed a decrease in activity. Cell populations treated with

anti-thy 1.2 and anti-asialo GM1 plus complement showed overall increases in activity. These results suggest that Amphotericin B stimulates macrophages for enhanced activity in the candidacidal assays.

Summary

The recent rise in fungal infections, and especially those that are life threatening, have been due to the increase in immunocompromised patients. Many researchers have acknowledged the importance of evaluating the effect of antifungal agents on host immunity, especially in the immunocompromised host. It is therefore interesting to note that none of the previously discussed research has been performed in an immunocompromised model. Knowing how antifungal agents interact with the immune system could be invaluable in selecting the appropriate agent for the treatment of a particular disease in a particular immunocompromised patient. The right choice of an antifungal agent for therapy could determine whether or not the treatment is successful and could make the difference between life and death in some patients.

MATERIALS AND METHODS

T-and B-Lymphocyte Proliferation Assays

Male Balb/c mice, 9 - 10 weeks of age, were obtained from Simonsen Laboratories, Gilroy, California, and housed in shoe box cages with corn-cob bedding. Mice were housed at a maximum of 5 mice per box, were fed Rodent Blox (Wayne Pet Food Division, Chicago, Illinois) and given water *ad libitum*. Some animals received a single 200 mg/kg I.P. dose of cyclophosphamide (Sigma Chemical Company, St. Louis, Missouri) 7 - 8 days prior to spleen removal for *in vitro* testing. Other animals received 25 mg/kg cyclosporine (SandImmune; Sandoz Pharmaceuticals Corporation, East Hanover, New Jersey) I.P. at 24 and 48 hours prior to spleen removal for *in vitro* testing.

On the day of *in vitro* testing, mice were euthanized with CO_2, and the spleens were removed aseptically and placed into centrifuge tubes containing RPMI-1640 (RPMI) medium (GIBCO Laboratories, Chagrin, Ohio). Spleens, pooled from 5-10 animals, were placed in a sterile whirlpak bag (Consolidated Plastics Company Inc., Twinsburg, Ohio) containing 5 - 10 mls of RPMI. The spleens were cut in half and rolled, using a rubber printing roller, until the spleen cells were pressed out. The cells were washed twice in 10 ml of RPMI, and the red blood cells were lysed by hypotonic lysis in which the cells were suspended in 9 ml distilled water for no longer than 15 seconds, after which 1 ml of 10X phosphate buffered saline (PBS) was added. The cells were centrifuged and resuspended in 5 ml of RPMI-1640 containing 2-mercaptoethanol (Sigma Chemical Company), sodium pyruvate (GIBCO Laboratories), and 20% fetal bovine serum (FBS; Hyclone Laboratories, Logan, Utah) (mouse media). The cells (>95% viability) were counted on a hemacytometer and diluted to appropriate concentrations in mouse media.

Ketoconazole (Sigma Chemical Company), Fluconazole, (Pfizer Ltd., Sandwich, Kent, United Kingdom) and Itraconazole (Janssen Research and Development, New Brunswick, New Jersey) were dissolved in acidified polyethylene glycol (PEG; J.T. Baker Chemical Company, Phillipsburg, New Jersey) and diluted immediately in RPMI containing 2% PEG. Concentrations of

32, 16, 8, 4 and 2 μg/ml were made, which resulted in final concentrations of 16, 8, 4, 2, and 1 μg/ml after the drug was mixed with spleen cells. Media contained a final concentration of 1% PEG.

Mouse media used for T-cell proliferation studies contained 1:100 dilution of phytohemaglutinin (PHA) stock solution (GIBCO laboratories). Mouse media used for B-cell proliferation studies contained 5 μg/ml lipopolysaccharide (LPS; Sigma Chemical Co.). One hundred microliters of a spleen cell suspension containing 5 X 10^6 cells/ml and 100 μl of each drug dilution were pipetted into triplicate wells of a 96 well tissue culture plate (Falcon; Becton Dickinson Labware, Oxnard, California) and incubated for 24 hours at 37C in 5% CO_2. Each well was pulsed with 2 μl (2 μCi) of methyl-^{3}H-thymidine (New England Nuclear, Boston, Massachusetts), and the cells were incubated for an additional 24 hours. Following incubation the cells were harvested onto glass filter paper (Filtermat; Skatron, Sterling, Virginia), and the paper was dried for a minimum of 18 hours at room temperature. Disks were cut from the filter paper and placed in scintillation vials (Skatron) to which 2 ml of scintillation fluid (Scintiverse; Fisher Scientific, Santa Clara, California) were added. The vials were counted on a Packard Tri-Carb 1500 Liquid Scintillation Analyzer (Coulter, Hialeah, Florida) to obtain counts per minute (CPM).

Natural Killer-Cell Studies

Methods for immunesuppression of mice, spleen cell preparation and drug preparation were identical to those used for lymphocytes proliferation studies. Yac target cells from a Yac-1 cell line (American Type Culture Collection, Rockville, Maryland), a Moloney Leukemia virus-induced mouse lynphoma, were grown in mouse media at 37C in 5% CO2. Approximately 1 X 10^7 cells in 10 ml of RPMI medium were centrifuged and all but 0.1 ml of the supernatant was pipetted off. Cells were labeled by adding 100 μl (100 μCi) of ^{51}Sodium Chromate (^{51}Cr; New England Nuclear) and incubating for 1 hour at 37C in 5% CO2. After incubation, the Yac cells were resuspended in 5 ml FBS and centrifuged to remove unincorporated label. The FBS was carefully removed, and the Yac cels were suspended in 5 ml of mouse media. The cells were counted on a hemacytometer and adusted to a concentration of 2 X 10^5 cells/ml.

Fifty microliters of Yac and spleen cell suspensions containing 1 X 10^4 and 1 X 10^6 cells respectively (effector cell:target cell ratio of 100:1) and 100 μl of each drug dilution were pipetted into triplicate wells of a 96-well tissue culture plate. Wells containing Yac cells only and Yac cells plus 5% saponin (Sigma Chemical Company) served as the spontaneous (lysis not associated with NK activity) and total release controls respectively. The plates were incubated for 4 hours at 37C in 5% CO_2, after which 0.1 ml of the supernatant was carefully pipetted from each well and placed in scintillation vials containing 2 ml of scintillation fluid. Vials were counted on a scintillation counter for CPM. Percent lysis was calculated by dividing CPM from the experiment lysis, minus the CPM in the spontaneous release control by the CPM of the total release control, minus the CPM in the spontaneous lysis control, and multiplying by 100.

Test results were analyzed using analysis of variance in a randomized block design. The experiments showing significant results ($P < 0.01$) were further analyzed using Fisher's least significant difference ($P < 0.01$) to determine concentrations that were significantly different from the controls. Experimental values from 3 experiments ($n=9$) for cell proliferation assays and from 2 experiments ($n=6$) for natural killer cell assays were combined for the analyses.

Lymphocyte Viability Assay

Mouse spleen cells, prepared as previously described, were stimulated with LPS and 5 X 10^5 cells were incubated in the presence of Ketoconazole, Itraconazole and Ketoconazole, prepared as described above, at final concentrations of 16, 8, 4, 2, 1 and 0 μg/ml for 48 hours at 37C in 5% CO_2. Following incubation spleen cell viability was determined by trypan blue exclusion.

RESULTS

Effects of Antifungal Agents on Cell Proliferation

Ketoconazole induced significant reductions in uptake of ^{3}[H]thymidine by T and B lymphocytes in almost all immune models and experiments. In T-cell proliferation studies Ketoconazole induced significant reductions as compared to drug free controls beginning at 1, 8 and 1 μg/ml in the normal, cyclophosphamide-compromised and cyclosporine-compromised models respectively (Fig. 1). In B-cell proliferation assays significant reductions began at 4, 8 and 4 μg/ml in the normal, cyclophosphamide-compromised and cyclosporine-compromised models respectively (Fig. 2). Starting at 1 μg/ml, Itraconazole significantly reduced uptake of ^{3}[H]thymidine by both T and B lymphocytes in all models (Fig. 3 and 4). Fluconazole had no significant effect on uptake of ^{3}[H]thymidine in either T- or B-cell proliferation studies in any of the immune models (Fig. 5 and 6).

Effects of Antifungal Agents on Natural Killer-Cell Activity

Ketoconazole had no significant effect on natural killer cell activity in any of the immune models (Fig. 7). Itraconazole induced significant reductions on natural killer cell activity in the immune-normal model beginning at 1 μg/ml. No effect was noted in the other immune models (Fig. 8). Fluconazole appeared to have no significant effect on natural killer-cell activity in any of the immune models (Fig. 9).

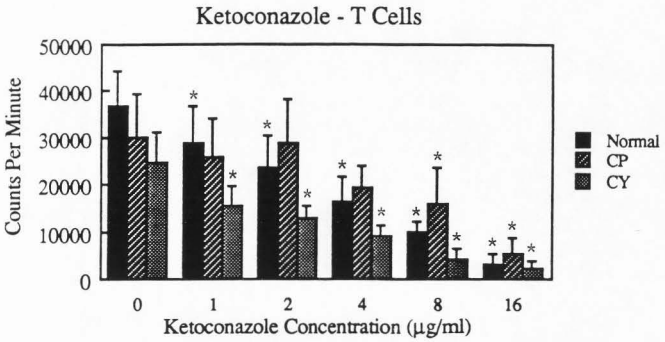

FIG. 1. Effect of ketoconazole concentration on uptake of ^{3}[H]-thymidine by T cells expressed as counts per minute in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

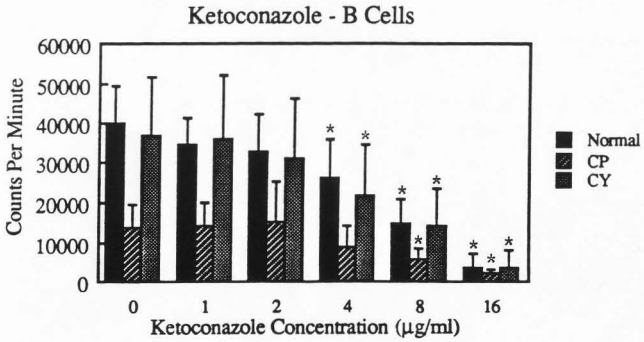

FIG. 2. Effect of ketoconazole concentration on uptake of ^{3}[H]-thymidine by B cells expressed as counts per minute in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

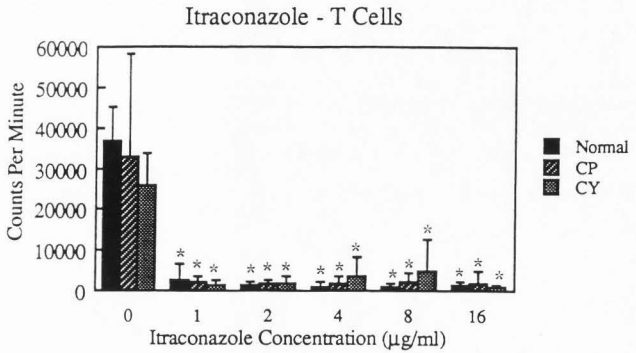

FIG. 3. Effect of itraconazole concentration on uptake of ^{3}[H]-thymidine by T cells expressed as counts per minute in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

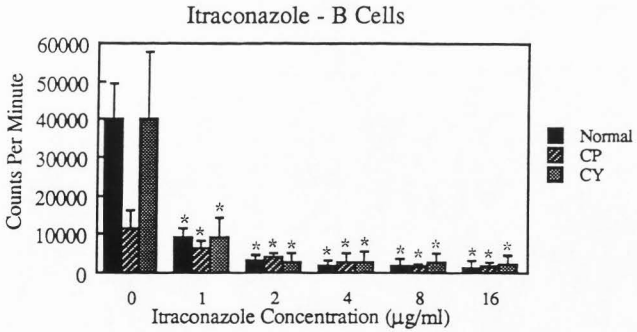

FIG. 4. Effect of itraconazole concentration on uptake of ^{3}[H]-thymidine by B cells expressed as counts per minute in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

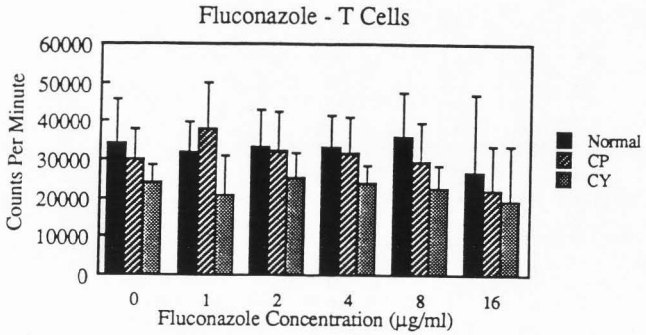

FIG. 5. Effect of fluconazole concentration on uptake of ^{3}[H]-thymidine by T cells expressed as counts per minute in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

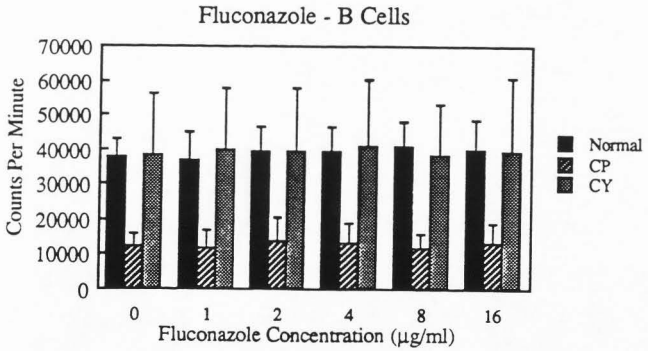

FIG. 6. Effect of fluconazole concentration on uptake of ^{3}[H]-thymidine by B cells expressed as counts per minute in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. Means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

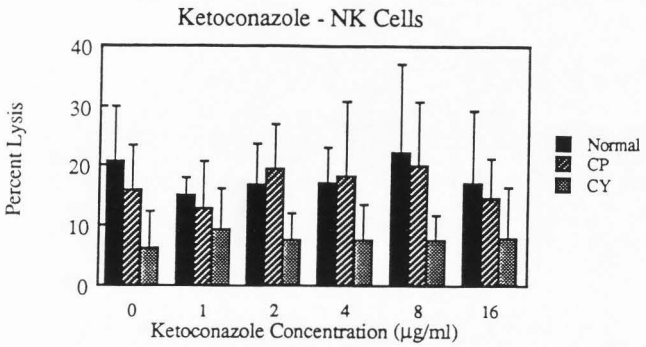

FIG. 7. Effect of ketoconazole concentration on lysis of Yac cells by natural killer cells in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

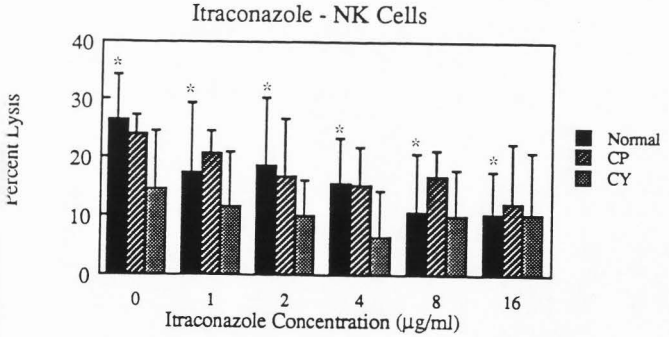

FIG. 8. Effect of itraconazole concentration on lysis of Yac cells by natural killer cells in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant difference ($P < 0.01$) from the control.

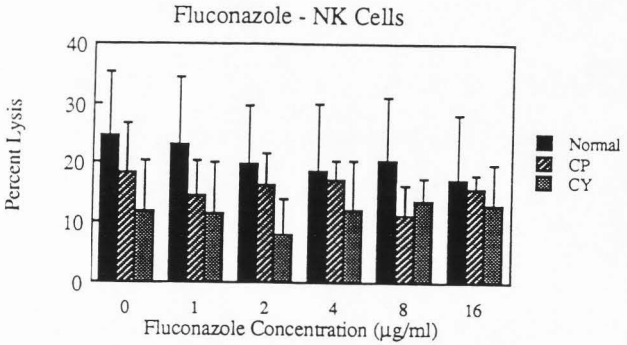

FIG. 9. Effect of fluconazole concentration on lysis of Yac cells by natural killer cells in normal, cyclosporine-compromised (CY) and cyclophosphamide-compromised (CP) immune models. The means and standard deviations are shown. An asterisk (*) above a column indicates a significant diference ($P < 0.01$) from the control.

Effects of Antifungal Agents on Lymphocyte Viability

Viability counts on LPS-stimulated lymphocytes following 2 days of incubation at 37C in 5% CO_2 in the presence of 0, 1, 2, 4, 8 and 16 µg/ml of Ketoconazole, Itraconazole and Fluconazole showed that Itraconazole killed 85% of lymphocytes at 1 µg/ml levels *in vitro* with up to a 98% kill at the16 µg/ml level. Lymphocytes incubated in the presence of Ketoconazole had viability counts of 78% of controls at the 1 µg/ml level with only 15% of controls viable at the 16 µg/ml level. Fluconazole did not effect overall live counts which ranged from 87% - 97% of controls (Table 1).

TABLE 1. Effect of ketoconazole, itraconazole and fluconazole concentration on viability of LPS stimulated lymphocytes. Values are shown as percentage of viable cells in comparison with controls.

	Concentration (μg/ml)				
Drug	1	2	4	8	16
Ketoconazole	78%	83%	68%	47%	15%
Itraconazole	15%	5%	1%	1%	2%
Fluconazole	87%	92%	93%	90%	97%

DISCUSSION

It would appear that Ketoconazole can be inhibitory to T- and B- cell proliferation at concentrations attainable in serum (3.5 μg/ml). Ketoconazole also has a more inhibitory effect against T cells which are approximately 4-fold more sensitive to this agent than B cells. Ketoconazole also appears to have a more profound effect on both T and B cells in the untreated and Cyclosporine-treated animals than that which occurs in the Cyclophosphamide-treated animals. These findings of the effects of Ketoconazole on T- and B-cell proliferation confirm the findings of experiments performed by Buttke and Chapman (12), Senior and Shaw (22) and Gergely et al. (16).

Itraconazole induces significant inhibitory effects on both T- and B-cell proliferation at approximate levels to those attainable in serum (0.6 μg/ml) and in tissues (up to 8 μg/ml). Experimental data from Abruzzo et al. (1) and Johnson et al. (17) show no significant reductions in the assays they performed, although these assays were not similar to neither T- and B-cell proliferation nor natural killer-cell assays.

Fluconazole does not appear to have any effects on the proliferation of T or B cells at concentrations attainable in serum (1 μg/ml). These conclusions are also supported by the work of Senior and Shaw (22).

The effects of Ketoconazole, Itraconazole and Fluconazole on lymphocyte-cell viability correlate well with the reductions in cell proliferation. Low lymphocyte viability suggests that the reductions in cell proliferation are a toxic effect and not just an inhibition of cell division. Itraconazole's profound effect on murine T- and B-lymphocyte proliferation, while other researchers have found that Itraconazole has little if any effect on cholesterol synthesis in human and rat subjects, may suggest another inhibitory mechanism than that of blocking cholesterol biosynthesis (12).

It does not appear that Ketoconazole or Fluconazole, at normal attainable levels, have any effect on natural killer-cell activity. It is interesting to note that Itraconazole induced significant reductions in percent lysis starting at 1 μg/ml in the immune-normal model but had no effect on percent lysis in the immune-compromised models. These results are contradictory with those of

Gergely et al. (16) who found that Ketoconazole at levels above 1 μg/ml inhibited natural killer-cell activity. This is probably due to the difference in the effector (human vs. murine) and target (Yac vs. K 562) cells used.

Some questions are raised as to why Ketoconazole and Itraconazole do not inhibit natural killer-cell activity to the same degree as they do lymphocyte proliferation. Some possible explanations are that these drugs have a more toxic effect on proliferating cells than on non-proliferating cells, as in the case of many antibiotics such as penicillin. Another possible explanation is that the natural killer assay is much shorter (4 hours) than the lymphocyte proliferation assays (48 hours). Longer exposure to the drug may be required to inhibit natural killer-cell activity.

Even though cyclophosphamide inhibits growth of all lymphocytes, it appears to preferentially inhibit B cells whose thymidine incorporation were considerably less than the controls. Cyclosporine, on the other hand, has little or no effect on cell proliferation but significantly reduces natural killer-cell activity.

Studies such as this one may be useful in determining which antifungal therapy would be most appropriate for patients undergoing a particular therapy that results in immunosuppression. It could be used to weigh a certain antifungal agent's efficacy in the treatment of a particular disease against any detrimental effect it may have on the immune system. As immunology and antifungal development are still very dynamic fields, the full ramifications of the immunological effects of antifungals is yet to be determined. This knowledge may make the difference between success or failure of antifungal treatment, which in turn may make the difference between life and death in cases of life-threatening mycosis.

Ketoconazole induces significant inhibitory effects on T- and B-cell proliferation in immune-normal and cyclosporine-compromised models, and has no significant effect on natural killer-cell activity at concentrations normally attainable in serum. Itraconazole induces significant inhibitory effects on T and B cells in all immune models, and also induces significant inhibitory effects on natural killer-cell activity in the immune-normal models at approximate levels attainable in serum

and at levels attainable in tissue. Fluconazole has no significant effects on neither T- and B-lymphocyte proliferation nor on natural killer-cell activity.

While *in vitro* results cannot be directly applied to *in vivo* situations, it may be possible to use *in vitro* testing to aid in screening compounds that may have detrimental effects on the immune system. Knowledge of the interaction of antifungal agents with the immune system is likely to become more increasingly important as the number of severely immune-compromised patients continues to be on the rise.

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APPENDIX

Experimental Data

The following tables give the experimental results for both cell proliferation studies and natural killer cell assays.

Cell Proliferation - Experiment #1

TABLE 2. Uptake of ^{3}[H]-thymidine expressed as counts per minute for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	41740	30430	28672	17522	11848	452
	Obs. #2	37406	28756	25418	15488	8540	230
	Obs. #3	36180	38298	24976	18798	8226	2030
	Mean	38442	32495	26355	17269	9538	904
	S.D.	2921	5095	2018	1669	2007	981
CY	Obs. #1	20798	12506	10814	6278	2460	602
	Obs. #	21694	14128	7882	5640	1788	3272
	Obs. #3	24314	15388	10960	7182	1908	334
	Mean	22269	14007	9885	6367	2052	1403
	S.D.	1827	1445	1736	775	358	1624
CP	Obs. #1	32278	30970	34710	23156	10400	2432
	Obs. #2	33000	27526	21856	19260	16188	1550
	Obs. #3	41106	29588	28900	27712	10586	2190
	Mean	35461	28361	28489	23376	12391	2057
	S.D.	4902	1733	6437	4230	3289	456

TABLE 3. Uptake of ^{3}[H]-thymidine expressed as counts per minute for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	37980	32660	26426	18682	6684	942
	Obs. #2	27870	25424	23208	19994	8618	844
	Obs. #3	34944	27128	27124	18146	11948	346
	Mean	33598	28404	28619	18941	9083	711
	S.D.	5188	3783	3213	951	2663	320
CY	Obs. #1	16926	13036	12350	6704	3246	216
	Obs. #2	15896	-	11396	6066	2386	400
	Obs. #3	19826	14710	9504	5592	2078	96
	Mean	17549	13873	11083	6121	2570	237
	S.D.	2038	1184	1449	558	605	153
CP	Obs. #1	14196	14712	10330	11958	5388	2058
	Obs. #2	11614	14878	10964	9848	4156	2142
	Obs. #3	11962	14050	9958	-	4566	1424
	Mean	12591	14547	10417	10904	4703	1875
	S.D.	1401	438	509	1491	627	393

TABLE 4. Uptake of ^{3}[H]-thymidine expressed as counts per minute for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	35002	136	1520	482	58	132
	Obs. #2	41654	404	1278	310	266	110
	Obs. #3	37374	198	1564	60	96	566
	Mean	38010	246	1454	284	140	269
	S.D.	3371	140	154	212	111	257
CY	Obs. #1	28918	368	166	148	354	1216
	Obs. #2	20410	416	1152	708	2160	270
	Obs. #3	16356	250	238	244	-	270
	Mean	21895	345	519	367	1257	585
	S.D.	6411	85	550	299	1277	546
CP	Obs. #1	30948	3692	1176	732	2510	456
	Obs. #2	38548	3604	3386	1336	1744	910
	Obs. #3	-	3752	1330	1116	908	614
	Mean	34748	3683	1964	1061	1721	660
	S.D.	5374	74	1234	306	801	230

TABLE 5. Uptake of ^{3}[H]-thymidine expressed as counts per minute for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	40310	8054	832	406	426	528
	Obs. #2	40236	7492	3990	482	1476	622
	Obs. #3	37672	6566	2404	256	2750	274
	Mean	39406	7371	2409	381	1551	475
	S.D.	1502	750	1579	115	1164	180
CY	Obs. #1	17650	2524	680	1002	708	414
	Obs. #2	17066	4130	804	334	250	176
	Obs. #3	19552	2378	490	422	382	216
	Mean	18089	3011	658	586	447	269
	S.D.	1300	972	158	363	236	127
CP	Obs. #1	11642	7924	3990	482	1476	622
	Obs. #2	9956	5094	2898	1858	1018	728
	Obs. #3	13732	6012	3302	888	940	618
	Mean	11777	6343	2849	1488	952	656
	S.D.	1892	1444	480	524	61	62

TABLE 6. Uptake of ^{3}[H]-thymidine expressed as counts per minute for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	41446	38754	42030	38070	31880	3522
	Obs. #2	39178	39376	40232	32552	31530	6004
	Obs. #3	38152	39838	35226	38498	27092	4234
	Mean	39592	39323	39163	36373	30167	4587
	S.D.	1686	544	3526	3316	2669	1278
CY	Obs. #1	21332	4226	20170	21610	15774	1922
	Obs. #2	22958	14670	21816	22162	15828	2128
	Obs. #3	24270	24032	28520	19148	17682	1776
	Mean	22853	14309	23502	20973	16428	1942
	S.D.	1472	9908	4423	1605	1086	177
CP	Obs. #1	45276	40764	44192	44506	10400	11730
	Obs. #2	33348	47492	43934	34510	29138	8612
	Obs. #3	25434	49676	31058	36276	32422	13470
	Mean	34686	45977	39728	38431	23987	11271
	S.D.	9988	4645	7510	5335	11880	2461

TABLE 7. Uptake of ^{3}[H]-thymidine expressed as counts per minute for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	36680	34066	34994	37716	41778	25962
	Obs. #2	34560	33666	28260	37190	38350	44084
	Obs. #3	38716	21728	37478	27690	37216	38470
	Mean	36652	29820	33577	34199	39115	36172
	S.D.	2078	7011	4770	5643	2375	9277
CY	Obs. #1	15202	13872	16260	14224	19684	13068
	Obs. #2	15524	17418	13448	17738	21252	14730
	Obs. #3	17124	18482	16752	17240	14848	16690
	Mean	15950	16591	15487	16401	18595	14829
	S.D	1029	2413	1783	1901	3338	1813
CP	Obs. #1	15564	13326	10074	13840	11076	13734
	Obs. #2	13784	4810	18428	13242	16160	13784
	Obs. #3	12978	11022	18170	12906	10946	14820
	Mean	14109	9719	15557	13329	12727	14113
	S.D.	1323	4405	4750	473	2973	613

Cell Proliferation - Experiment #2

TABLE 8. Uptake of ^{3}[H]-thymidine expressed as counts per minute for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in an immune normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	29190	17462	16320	11580	7062	3806
	Obs. #2	22598	23906	14174	12814	5866	2178
	Obs. #3	41600	16302	16776	8266	12708	2952
	Mean	31129	19223	15757	10887	8545	2979
	S.D.	9648	4097	1389	2352	3654	814
CY	Obs. #1	20016	11808	11964	13080	4350	1342
	Obs. #2	19980	11918	12422	9468	4228	4054
	Obs. #3	30082	14322	16856	9252	4348	1140
	Mean	23359	12683	13747	10600	4309	2179
	S.D.	5822	1421	2702	2150	70	1627
CP	Obs. #1	25582	17872	21228	14286	5880	7822
	Obs. #2	-	20700	22738	17592	20044	4934
	Obs. #3	24270	16124	27356	23972	31186	2438
	Mean	24926	18232	23774	18617	19037	8398
	S.D.	927	2309	3193	4924	12683	3785

TABLE 9. Uptake of ^{3}[H]-thymidine expressed as counts per minute for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in an immune normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	34499	31097	38511	28365	13375	4705
	Obs. #2	36995	32515	34109	21473	13553	2565
	Obs. #3	37341	34087	32619	18873	10847	1911
	Mean	36278	32566	35080	22904	12592	3060
	S.D.	1551	1496	3064	4905	1514	1461
CY	Obs. #1	42655	47615	35793	27101	15245	3993
	Obs. #2	50217	40317	40811	23443	18271	2899
	Obs. #3	46285	38711	38025	26599	16461	2829
	Mean	46366	42214	38210	25714	16659	3240
	S.D.	3782	4746	2514	1983	1523	653
CP	Obs. #1	5855	6637	9703	3907	2719	1497
	Obs. #2	8555	5919	8817	6153	3635	1561
	Obs. #3	9177	9681	7631	4883	3997	1355
	Mean	7862	7412	8717	4981	3450	1471
	S.D.	1766	1997	1040	1126	659	105

TABLE 10. Uptake of ^{3}[H]-thymidine expressed as counts per minute for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in an immune normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	26022	2388	288	814	1458	2452
	Obs. #2	32916	12712	1264	536	2168	706
	Obs. #3	36098	2446	346	340	694	1380
	Mean	31679	5849	633	563	1440	1513
	S.D.	5151	5944	548	238	737	881
CY	Obs. #1	14488	670	1002	366	5622	160
	Obs. #2	28700	4060	3996	3850	1170	1616
	Obs. #3	24072	2788	1830	7630	2032	212
	Mean	22420	2506	2276	3949	2941	663
	S.D.	7249	1713	1546	3633	2361	826
CP	Obs. #1	15180	962	952	954	4022	1182
	Obs. #2	5962	2064	1946	6144	1488	1766
	Obs. #3	25452	1832	2612	900	7154	2414
	Mean	15531	1619	1837	2666	4221	1787
	S.D.	9750	581	835	3012	2836	616

TABLE 11. Uptake of ^{3}[H]-thymidine expressed as counts per minute for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in an immune normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	27093	10729	4985	1527	1041	1607
	Obs. #2	34591	10455	4035	1873	791	1593
	Obs. #3	31501	14673	5087	745	1305	1627
	Mean	31062	11952	4702	1382	1046	1609
	S.D.	3768	2360	580	578	257	17
CY	Obs. #1	46861	14983	3877	979	1223	1661
	Obs. #2	53359	12837	4939	9257	6151	2099
	Obs. #3	51629	14529	2729	4573	4212	1779
	Mean	50616	14116	3848	4573	4121	1779
	S.D.	3365	1131	1105	4245	2576	280
CP	Obs. #1	5173	6283	6517	2721	2299	2941
	Obs. #2	4463	5695	4159	3367	1467	2835
	Obs. #3	11273	5107	3243	1533	1045	1337
	Mean	6970	5695	4640	2540	1604	2371
	S.D.	3744	588	1689	930	638	897

TABLE 12. Uptake of ^{3}[H]-thymidine expressed as counts per minute for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in an immune normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	29462	21836	22952	21392	28280	28916
	Obs. #2	19666	22628	21906	40208	24256	20728
	Obs. #3	25644	21550	26484	17716	31602	30478
	Mean	24924	22005	23781	26439	28046	26707
	S.D.	4938	558	2399	12065	3679	5237
CY	Obs. #1	19156	19906	14414	22288	20326	22792
	Obs. #2	21510	22144	23116	19742	24606	26998
	Obs. #3	25492	9328	27904	24332	24424	34132
	Mean	22063	17126	21811	22121	23119	27974
	S.D.	3203	6845	6839	2300	2420	5733
CP	Obs. #1	22074	20360	14788	12452	22036	18862
	Obs. #2	25390	33320	20630	31024	25238	15422
	Obs. #3	20154	22392	25628	28400	31186	25218
	Mean	22539	25357	20349	23959	26153	19834
	S.D.	2649	6970	5425	10051	4643	4970

TABLE 13. Uptake of ^{3}[H]-thymidine expressed as counts per minute for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in an immune normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	38711	36627	39379	33681	31885	36203
	Obs. #2	30249	33867	35259	41403	32699	41205
	Obs. #3	31961	33419	37045	35711	39613	30045
	Mean	33640	34638	37228	36932	34732	35818
	S.D.	4474	1737	2066	4003	4246	5590
CY	Obs. #1	39709	59683	53043	58801	43649	43573
	Obs. #2	42429	48671	48049	46027	50571	36643
	Obs. #3	54551	46225	51197	59291	45603	43301
	Mean	45563	51526	50763	54706	46608	41172
	S.D.	6452	5853	2062	6140	2914	3205
CP	Obs. #1	8059	8213	6159	6059	8729	5493
	Obs. #2	6737	7871	7911	6089	6367	5983
	Obs. #3	6165	6197	7453	7941	7227	7717
	Mean	6987	7427	7174	6696	7441	6398
	S.D.	793	881	742	880	976	954

Cell Proliferation - Experiment #3

TABLE 14. Uptake of ^{3}[H]-thymidine expressed as counts per minute for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	33768	36664	26248	24584	9840	5698
	Obs. #2	39246	31860	24854	16828	11426	5830
	Obs. #3	48210	25242	35464	21506	11836	4664
	Mean	40408	34589	28855	20973	11034	5397
	S.D.	7291	2468	5766	3905	1054	639
CY	Obs. #1	21380	15460	15272	9132	7654	5056
	Obs. #2	24886	20996	15010	10322	6644	1688
	Obs. #3	39640	23440	13772	10646	5686	1502
	Mean	28635	19965	14685	10033	6661	2749
	S.D.	9690	4089	801	797	984	2000
CP	Obs. #1	30452	22698	46578	14234	19854	4870
	Obs. #2	31524	25268	18146	16424	9048	5202
	Obs. #3	41262	42758	37942	17788	19052	6300
	Mean	34413	30241	34222	16149	15985	5457
	S.D.	5956	10916	14576	10033	6661	2749

TABLE 15. Uptake of ^{3}[H]-thymidine expressed as counts per minute for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	50904	41192	43104	45554	19806	1470
	Obs. #2	58900	41882	45746	38630	28360	4134
	Obs. #3	39948	45230	-	27170	17276	5484
	Mean	49917	42768	44425	37118	21814	7029
	S.D.	9514	2160	1868	9285	5808	3905
CY	Obs. #1	46704	53158	38870	39346	21172	3454
	Obs. #2	50122	53610	45110	28540	24906	4486
	Obs. #3	41736	25414	46194	34132	23884	4334
	Mean	46187	44061	43391	34006	23321	7425
	S.D.	4217	16150	3953	5404	1930	6006
CP	Obs. #1	21524	18482	23934	16610	8470	3424
	Obs. #2	17794	18684	17002	14068	10224	2896
	Obs. #3	22524	23786	38678	12358	8634	3422
	Mean	20614	20321	26538	14345	9109	3247
	S.D.	2493	3003	11070	2140	969	304

TABLE 16. Uptake of ^{3}[H]-thymidine expressed as counts per minute for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	35834	776	3598	4462	486	878
	Obs. #2	32438	1468	1392	112	1602	1566
	Obs. #3	-	1276	358	328	1904	2870
	Mean	34136	1173	1783	1634	1331	1772
	S.D.	2401	357	1655	2451	747	1012
CY	Obs. #1	37540	2426	5196	-	2504	1066
	Obs. #2	25324	1068	742	1178	1848	288
	Obs. #3	36014	1156	872	1388	1228	1286
	Mean	32959	1550	2270	1283	1860	880
	S.D.	6656	760	2535	148	638	524
CP	Obs. #1	57256	2086	1472	2530	1374	9028
	Obs. #2	40994	166	1730	1104	238	288
	Obs. #3	80800	2148	2656	608	74	946
	Mean	59683	1467	1953	1414	95	3421
	S.D.	20014	1127	623	998	542	4867

TABLE 17. Uptake of ^{3}[H]-thymidine expressed as counts per minute for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	45172	7652	3164	2242	1338	840
	Obs. #2	50778	9672	3388	2488	1582	1446
	Obs. #3	55618	6500	3040	4819	6622	5252
	Mean	50523	7941	3197	3183	3181	2513
	S.D.	5228	1606	176	1422	2983	2392
CY	Obs. #1	56422	10274	2670	1266	3792	5480
	Obs. #2	44120	8984	6096	4268	4956	2600
	Obs. #3	56356	11626	4692	3086	3666	6036
	Mean	52299	10295	4486	2873	4138	4705
	S.D.	7084	1321	1722	1512	711	1844
CP	Obs. #1	14860	5490	5030	2212	2186	1734
	Obs. #2	18334	9618	3654	-	1660	1308
	Obs. #3	15370	8798	4590	1580	3248	3430
	Mean	16188	7969	4425	1896	2365	2157
	S.D.	1876	2185	703	446	809	1123

TABLE 18. Uptake of ^{3}[H]-thymidine expressed as counts per minute for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--T cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	17056	33236	27674	41618	46970	51640
	Obs. #2	43386	34342	35480	34004	39420	35654
	Obs. #3	51462	35982	49168	33522	60976	59372
	Mean	37301	34520	37441	36381	49122	48889
	S.D.	14690	1128	8884	3708	8931	9876
CY	Obs. #1	19816	30818	29144	26530	28472	23840
	Obs. #2	25660	24014	27736	23134	24052	21138
	Obs. #3	34950	36464	36014	35576	32818	39390
	Mean	26809	30432	30965	28413	28447	28123
	S.D.	7632	6234	4429	6413	4383	9851
CP	Obs. #1	34088	56196	37946	26888	29350	30458
	Obs. #2	32896	31014	31902	31590	36076	43888
	Obs. #3	31168	39022	38906	40414	48592	32428
	Mean	32717	42077	36251	32964	38006	35591
	S.D.	1199	10505	3100	5607	7973	5921

TABLE 19. Uptake of ^{3}[H]-thymidine expressed as counts per minute for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models--B cells

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	39106	49696	48400	37959	50214	52940
	Obs. #2	47480	45572	46238	51770	47128	47022
	Obs. #3	43008	42700	49288	49632	50246	43928
	Mean	43198	45989	47975	46454	49196	47963
	S.D.	4190	3517	1569	7434	1791	4579
CY	Obs. #1	55904	51200	52150	54438	52040	55302
	Obs. #2	51992	56068	48922	55552	50778	52082
	Obs. #3	53094	47642	55824	46240	44336	77358
	Mean	53663	51637	52299	52077	49051	61581
	S.D.	2017	4230	3453	5085	4132	13758
CP	Obs. #1	14552	17688	12666	17582	15336	17808
	Obs. #2	15134	19028	21736	20270	13386	20658
	Obs. #3	15466	17104	23424	20504	17256	18418
	Mean	15051	17940	19275	19452	15326	18961
	S.D.	463	986	5786	1624	1935	1501

Natural Killer Cell Assay
Experiment #1

TABLE 20. Percent lysis for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	18.24	13.07	16.99	24.78	18.21	9.23
	Obs. #2	16.85	13.08	14.19	12.56	15.36	15.17
	Obs. #3	14.69	17.48	10.36	16.05	17.52	16.81
	Mean	16.60	14.54	13.85	17.80	17.03	13.74
	S.D.	1.79	2.54	3.33	6.29	1.48	3.98
CY	Obs. #1	7.07	20.12	4.49	12.98	5.66	2.77
	Obs. #2	-2.21	10.66	10.15	8.05	7.20	6.80
	Obs. #3	13.16	0.68	8.46	6.5	2.80	0.14
	Mean	6.01	10.49	7.70	9.17	5.22	3.23
	S.D.	7.74	9.72	2.90	3.38	2.23	3.36
CP	Obs. #1	14.85	11.83	12.32	14.34	5.28	15.23
	Obs. #2	19.17	22.68	10.44	-0.82	21.33	4.23
	Obs. #3	11.07	19.00	22.01	19.79	21.22	14.65
	Mean	15.03	17.84	14.92	11.10	15.94	11.37
	S.D.	4.05	5.52	7.70	10.68	9.23	6.19

TABLE 21. Percent lysis for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	17.10	11.94	7.44	7.24	6.05	5.29
	Obs. #2	18.98	-	8.50	12.31	1.52	8.44
	Obs. #3	23.26	15.68	17.95	14.85	3.53	2.86
	Mean	19.78	13.81	11.30	11.46	3.70	5.53
	S.D.	3.16	2.64	5.79	3.87	2.27	2.80
CY	Obs. #1	8.47	3.04	5.84	2.69	3.83	3.27
	Obs. #2	3.61	7.86	6.48	6.34	6.98	3.39
	Obs. #3	9.95	7.15	1.79	4.09	2.54	2.94
	Mean	7.34	6.02	4.71	4.38	4.45	3.20
	S.D.	3.32	2.60	2.54	1.84	2.28	0.24
CP	Obs. #1	28.54	18.98	22.21	14.31	16.40	-
	Obs. #2	21.36	22.73	-	5.68	10.60	12.38
	Obs. #3	19.85	16.00	-1.02	15.10	12.37	15.41
	Mean	23.25	19.24	10.56	15.03	13.12	13.90
	S.D.	4.64	3.37	16.42	0.69	2.97	2.14

TABLE 22. Percent lysis for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	17.31	18.00	13.39	9.52	12.49	10.12
	Obs. #2	18.10	7.67	16.02	11.20	16.00	13.13
	Obs. #3	14.34	16.82	9.31	16.63	12.96	12.67
	Mean	16.59	14.16	12.90	12.45	13.82	11.97
	S.D.	1.98	5.66	3.38	3.71	1.91	1.62
CY	Obs. #1	9.54	7.21	8.38	8.47	11.68	8.21
	Obs. #2	8.49	0.35	3.80	-0.50	7.93	7.62
	Obs. #3	-0.61	7.61	-1.83	7.88	10.82	6.92
	Mean	5.81	5.06	3.45	5.28	10.14	7.59
	S.D.	5.58	4.08	5.11	5.02	1.97	0.65
CP	Obs. #1	11.53	15.97	20.09	13.08	14.19	13.69
	Obs. #2	28.25	17.74	22.48	22.59	5.75	17.05
	Obs. #3	26.18	21.97	18.31	14.56	19.93	19.53
	Mean	21.99	18.56	20.30	16.74	13.29	16.76
	S.D.	9.11	3.08	2.09	5.12	7.13	2.93

Natural Killer Cell Assay
Experiment #2

TABLE 23. Percent lysis for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	62.68	56.30	61.72	47.37	47.37	58.21
	Obs. #2	57.58	54.07	53.11	63.64	43.86	93.94
	Obs. #3	39.07	44.18	66.83	62.36	50.88	42.90
	Mean	53.11	51.52	60.55	57.79	47.37	65.02
	S.D.	12.42	6.45	6.93	9.05	3.51	26.19
CY	Obs. #1	18.02	35.89	37.80	66.19	27.91	37.48
	Obs. #2	39.71	44.50	20.26	18.02	21.53	37.80
	Obs. #3	15.79	30.14	19.30	29.51	40.03	14.51
	Mean	24.51	36.84	25.78	37.91	29.82	29.93
	S.D.	13.21	7.22	10.42	25.16	9.40	13.35
CP	Obs. #1	62.36	51.52	52.15	44.18	45.45	41.63
	Obs. #2	24.08	19.30	21.63	0.80	20.89	29.19
	Obs. #3	40.67	15.79	33.01	46.73	30.46	45.77
	Mean	42.37	28.87	35.57	30.57	32.27	38.86
	S.D.	19.20	19.69	15.47	25.81	12.38	8.63

TABLE 24. Percent lysis for itraconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	51.52	43.54	53.43	35.25	48.64	39.39
	Obs. #2	40.67	62.36	59.49	43.86	47.37	50.88
	Obs. #3	45.77	27.27	24.40	46.73	62.04	30.46
	Mean	45.99	44.39	45.77	41.95	52.68	40.24
	S.D.	5.43	17.56	18.75	5.98	8.13	10.23
CY	Obs. #1	37.80	43.54	27.27	51.20	24.72	40.67
	Obs. #2	37.16	41.31	37.48	39.39	48.96	22.17
	Obs. #3	15.47	25.68	29.82	21.53	29.19	55.98
	Mean	30.14	36.84	31.53	37.37	34.29	39.61
	S.D.	12.71	9.72	5.31	14.94	12.90	16.93
CP	Obs. #1	62.68	33.01	36.20	33.33	27.19	40.67
	Obs. #2	40.03	27.27	33.33	13.88	31.74	22.17
	Obs. #3	34.61	34.93	32.38	36.20	26.32	55.98
	Mean	45.77	31.74	33.97	27.80	28.65	39.61
	S.D.	14.89	3.98	1.99	12.15	2.79	16.93

TABLE 25. Percent lysis for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	56.30	57.26	59.49	57.58	61.72	63.96
	Obs. #2	59.49	58.53	74.48	55.66	44.18	28.55
	Obs. #3	-	56.94	48.96	53.11	36.84	54.07
	Mean	57.89	57.58	60.98	55.45	47.58	48.86
	S.D.	2.26	0.84	12.82	2.24	12.78	18.27
CY	Obs. #1	40.35	37.48	31.74	38.44	40.99	34.61
	Obs. #2	30.46	37.48	27.59	35.57	37.16	28.55
	Obs. #3	9.73	23.76	27.91	79.59	16.75	11.96
	Mean	26.85	32.91	29.08	51.20	31.63	25.04
	S.D.	15.63	7.92	2.31	24.63	13.03	11.72
CP	Obs. #1	13.56	57.26	27.27	55.66	65.87	43.86
	Obs. #2	49.28	58.53	42.90	42.58	44.82	16.11
	Obs. #3	30.14	56.94	43.54	-	44.50	13.24
	Mean	30.99	57.58	37.91	49.12	51.73	24.40
	S.D.	17.88	0.84	9.21	9.25	12.25	16.91

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TABLE 26. Percent lysis for ketoconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	37.15	19.29	28.30	21.68	48.16	37.48
	Obs. #2	16.51	11.95	14.90	11.17	11.67	6.83
	Mean	26.83	15.62	21.60	16.43	29.92	22.16
	S.D.	14.59	5.19	9.48	7.43	25.81	21.68
CY	Obs. #1	2.27	7.00	12.95	-1.91	9.17	22.29
	Obs. #2	10.61	8.28	1.82	12.17	13.84	8.44
	Mean	6.44	7.64	7.39	5.13	11.50	15.37
	S.D.	5.90	0.90	7.87	9.95	3.30	9.80
CP	Obs. #1	26.97	5.88	26.52	32.09	34.98	19.01
	Obs. #2	7.66	6.11	25.97	26.19	17.51	21.07
	Mean	17.32	5.99	26.24	29.14	26.24	20.04
	S.D.	13.65	0.16	0.39	4.17	12.35	1.46

TABLE 27. Percent lysis for itraconazole 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	30.66	36.17	36.08	17.65	12.32	18.86
	Obs. #2	37.20	19.72	22.82	29.97	28.68	20.07
	Obs. #3	31.00	-	-	11.28	12.14	5.86
	Mean	32.96	27.95	29.45	19.64	17.71	14.93
	S.D.	3.68	11.63	9.38	9.50	9.50	7.88
CY	Obs. #1	15.16	19.89	15.67	-0.43	18.60	27.47
	Obs. #2	31.95	26.18	14.98	21.70	21.53	13.95
	Obs. #3	18.69	5.86	15.76	4.56	6.29	-
	Mean	21.93	17.31	15.47	8.61	15.47	20.71
	S.D.	8.85	10.41	0.42	11.61	8.09	9.56
CP	Obs. #1	23.08	29.54	20.84	25.06	22.82	20.15
	Obs. #2	25.06	14.04	17.48	17.57	20.15	23.17
	Obs. #3	26.01	17.91	27.04	18.86	3.44	16.62
	Mean	24.72	20.50	21.79	20.50	15.47	19.98
	S.D.	1.49	8.07	4.85	4.01	10.50	3.28

TABLE 28. Percent lysis for fluconazole at 0, 1, 2, 4, 8 and 16 μg/ml in immune-normal, cyclosporine (CY) and cyclophosphamide (CP) immune-compromised models.

		Concentration (μg/ml)					
Model		0	1	2	4	8	16
Normal	Obs. #1	21.34	23.12	17.70	15.55	14.06	19.04
	Obs. #2	36.55	37.29	34.55	41.15	39.03	37.40
	Obs. #3	39.59	35.36	28.83	17.33	28.42	9.76
	Mean	32.49	31.92	27.03	24.68	27.17	22.40
	S.D.	9.78	7.69	8.57	14.30	12.14	14.62
CY	Obs. #1	16.44	22.30	12.60	20.00	18.29	17.40
	Obs. #2	25.34	20.82	13.62	20.30	15.25	15.62
	Obs. #3	12.06	11.61	10.87	16.59	16.96	23.19
	Mean	17.95	18.24	12.33	18.96	16.83	18.74
	S.D.	6.77	5.79	1.38	2.03	1.53	3.96
CP	Obs. #1	14.14	10.35	12.06	17.48	10.58	13.54
	Obs. #2	12.21	6.86	8.57	17.85	8.79	14.14
	Obs. #3	-	-	17.11	17.03	7.98	16.44
	Mean	13.18	8.61	12.58	17.45	9.12	14.71
	S.D.	1.37	2.47	4.29	0.41	1.33	1.53